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Preparation and characterization of NPs coated with different surface chemistries

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BBB pathophysiology–independent delivery of siRNA in traumatic brain injury

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Detailed protocol

siRNA-loaded PLGA NPs with different surface coatings were prepared by a modified nanoprecipitation method. Briefly, the organic phase was prepared by mixing 20 µl of siRNA (4 nmol in water) with 1 ml of PLGA (Durect Corporation) (5 mg/ml in acetone, DMF, or tetrahydrofuran) and 200 µl of the cationic lipid-like molecule (5 mg/ml), which was synthesized by reacting the ethylenediamine core PAMAM generation 0 dendrimer (Sigma-Aldrich) and 1,2 epoxytetradecane (Sigma-Aldrich) using a ring opening reaction, as described previously (*58*). The siRNA sequences used in this study include the following: luciferase siRNA: 5′-CUU ACG CUG AGU ACU UCG AdTdT-3′ (sense strand) and 5′-UCG AAG UAC UCA GCG UAA GdTdT-3′ (antisense strand); tau siRNA: 5′-CCU AGA AAU UCC AUG ACG AUU-3′ (sense strand) and 5′-UCG UCA UGG AAU UUC UAG GUU-3′ (antisense strand); scrambled siRNA: 5′-UUC UCC GAA CGU GUC ACG UUU-3′ (sense strand) and 5′-ACG UGA CAC GUU CGG AGA AUU-3′ (antisense strand); Cy3 or Dy677-labeled siRNAs were synthesized by labeling the 5′-end of both the sense and antisense strands of scrambled siRNA with Cy3 and Dy677. Under vigorous stirring, the mixture was added slowly into a 15-ml aqueous solution. Various coating materials were added to either the organic phase or water phase to endow the surface of NPs with different coatings. For PEG-NPs, the organic phase contained DSPE-PEG (2 mg/ml) (PEG molecular weight 3000) (Avanti Polar Lipids). For GSH-NPs, 5, 10, or 25 mole percent (mol %) of DSPE-PEG was replaced with DSPE-PEG-GSH to yield GSH (L)-NPs, GSH (M)-NPs, and GSH (H)-NPs, respectively. For Tf-NPs, 10 mol % of DSPE-PEG was replaced with DSPE-PEG-Tf. For F-68-NPs, the organic phase contained DSPE-PEG (1 mg/ml), and the water phase contained Pluronic F-68 (1 mg/ml) (Sigma-Aldrich). For PS 80 (L)-NPs, the organic phase contained DSPE-PEG (1.5 mg/ml), and water phase contained PS 80 (0.5 mg/ml) (Sigma-Aldrich); for PS 80 (M)-NPs, the organic phase contained DSPE-PEG (1 mg/ml), and water phase contained PS 80 (1 mg/ml); for PS 80 (H)-NPs, the water phase contained PS 80 (2 mg/ml). Concentrations of surface coating materials were based on previously published reports (*31*, *34*, *35*). PS 80-NPs and GSH-NPs used for evaluating the impact of surface coating chemistry on cellular uptake, gene silencing, and BBB penetration in healthy mice were formulated with medium coating densities of PS 80 and GSH. NPs with medium coating densities of PS 80 or GSH were prepared by using their respective solutions at concentrations that have been previously used to formulate BBB-penetrating NPs. Lower and higher concentrations compared to the one chosen from literature were then used to prepare NPs with low and high coating densities of PS 80 and GSH. The resulting NP dispersions were transferred to centrifuge filters with a 300-kDa molecular weight cutoff (MWCO) (Sartorius Vivaspin) and collected via centrifugation and washed with cold water three times. Last, they were dispersed in PBS. Particle size distribution and surface charge of NPs were measured by DLS (Brookhaven Instruments Corporation). NPs were stained with 1% uranyl acetate, and their morphologies and size were observed under the Tecnai G2 Spirit BioTWIN transmission electron microscope (FEI Company).

To determine siRNA encapsulation efficiency, Cy3-labeled scrambled siRNA–loaded NPs were prepared as described above. Five microliters of NP solution was removed and mixed with 95 µl of dimethyl sulfoxide (DMSO). Fluorescence intensity was analyzed using a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments). Release kinetics of siRNA from NPs was also evaluated using Cy3-labeled scrambled siRNA–loaded NPs. Suspension of NPs in PBS was placed in dialysis tubing (float-a-lyzer G2 dialysis device, MWCO 100 kDa, Spectrum Labs) and dialyzed against PBS (pH 7.4) at 37°C with a shaking speed of 150 rpm. At different time points, an aliquot of the NP suspension was withdrawn and dissolved in DMSO, and the fluorescence intensity of siRNA was analyzed. To determine PS 80 coating density on PS 80–coated NPs, PS 80-NP suspension was added to DMSO in a 1:10 ratio to dissolve the NPs and the concentration of PS 80 in the solution was analyzed using a high-performance liquid chromatography evaporative light scattering detector (Agilent 1260 Infinity II; Column: Zorbax 300SB-C18, 3 × 150 mm, 3.5 µm; flow rate: 1 ml/min; PS 80 peak elution time: 7.8 min in a water-acetonitrile gradient). To determine GSH coating density on NPs, GSH-NP suspension was concentrated by centrifugation and added to methanol in a 1:9 ratio. Concentration of GSH in the solution was analyzed using the Micro BCA (bicinchoninic acid) Protein Assay Kit (Thermo Fisher Scientific). Briefly, GSH was quantified from the dissolved GSH-NPs solution following 30-min incubation with BCA reagents at 37°C, in accordance with the manufacturer's protocol. All analyses were performed in triplicate.

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2. Li, W., Qiu, J., Li, X., Aday, S., Zhang, J., Conley, G., Xu, J., Joseph, J., Lan, H., Langer, R., Mannix, R., Karp, J. M. and Joshi, N.(2020). BBB pathophysiology–independent delivery of siRNA in traumatic brain injury. Science Advances 7(1). DOI: 10.1126/sciadv.abd6889